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# Ozone biomonitoring using tobacco, *Nicotiana tabacum* "BelW3"

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**Abstract.** The ozone sensitive tobacco cultivar BelW3 (*Nicotiana tabacum* L.) was used to monitor ozone pollution for one vegetation period (beginning of May till end of September) in Graz, a city of approximately 240 000 inhabitants in the south of Austria. The plants were exposed for 14 days in standardized shaded exposition racks. The insensitive tobacco cultivar BelB was used as a control. The leaf necroses characteristic for ozone damage were well correlated with ozone dose (AOT 40) with the exception of the first exposition period in May. The reliability of the evaluation of visible symptoms on tobacco leaves was confirmed by computer-aided image analysis. It was possible to identify the spatial and temporal ozone distribution within a project area.

**Key words:** Ozone biomonitoring, *Nicotiana tabacum* "BelW3", leaf damage, AOT 40

## Introduction

Bioindication permits a qualitative and quantitative assessment of the effect of anthropogenic and natural influences using suitable indicator organisms which react to environmental influences by changing their vital functions and/or chemical composition (VDI 1999). In the case of air-quality control the use of bioindicator plants is an appropriate means to detect and monitor air pollution effects (Klumpp & al. 2002).

The ozone sensitive tobacco cultivar BelW3 produces characteristic lesions as a response to ozone exposure (Heggestad 1991, Krupa & al. 1993, Nouchi 2002) and this visible foliar response is widely used in ozone monitoring projects (e.g., Arndt & al. 1987, Heggestad 1991, Klumpp & al. 2002, Nouchi 2002).

A monitoring study with plants as bioindicators was performed in Graz, a city of approximately 240 000 inhabitants in the south of Austria. The results of monitoring ozone using tobacco, *Nicotiana tabacum* "BelW3", are presented here. Apart from gathering information on air quality in different areas of the city the reliability of the visual estimation and the dependence of leaf damage on the ozone dose was investigated.

## Materials and Methods

Bioindicator plants were exposed 1996 on 11 sites in the city area of Graz (Fig. 1). The first exposition started 7th of May and the last exposition ended 24th of September.

The cultivar BelW3 was used as an ozone sensitive indicator. Tobacco cultivar BelB, an ozone tolerant variety, was used as a control. 6–8 weeks old plants were exposed on shaded and slightly elevated exposition racks in self watering assemblies for 14 days (ARNDT & al. 1987, VDI 2000, GROSS 2001). Then the percentage of damaged leave area was visually estimated and classified into damage classes (Tab. 1). The damage class of one plant resulted from adding up the damage classes of all leaves (average 8 leaves) and dividing by the number of leaves. The mean damage class of one site was the mean of all exposed plants (2–4).

On 4 sites (sites 1, 3, 5, 6) continuous measurements of ozone concentrations (half hour average means) were available and were used to determine the AOT 40 value (ppb.h; 00:00–24:00).

The reliability of the evaluation of visible symptoms on tobacco leaves was confirmed by computer-aided image analysis (Optimas 5.2). Percentage of injury was visually estimated and the leaves classified into damage classes (Tab. 1). Then the leaves were scanned, analysed and also classified into damage classes.

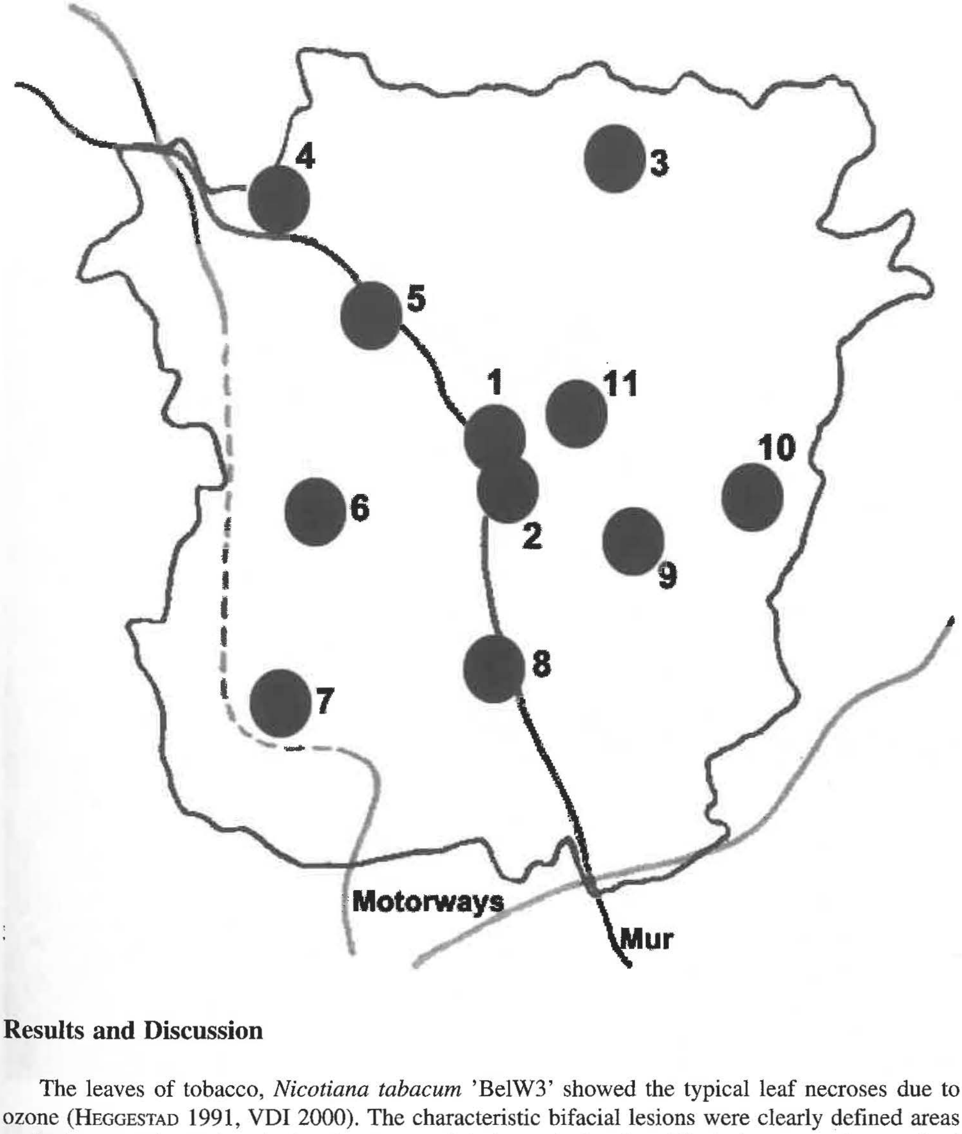
Other bioindicators (*Phaseolus vulgaris* L., *Trifolium subterraneum* L., *Medicago sativa* L. and *Lolium multiflorum* Lam.) were also exposed to monitor the effect and the distribution on $NO_x$, $SO_2$ and the accumulation of heavy metals (DUGAUQUIER & al. 1997, GROSS 2001, STABENTHEINER & al. 2002).

This presentation only focuses on monitoring ozone.

Table 1: Damage classes (*Nicotiana tabacum* 'BelW3') based on the percentage of foliar surface affected (according to ARNDT & al. 1987)

| Damage class | Percentage affected leaf area |
|---|---|
| 0 | 0 |
| 1 | 0–2 |
| 2 | 2–5 |
| 3 | 5–10 |
| 4 | 10–25 |
| 5 | 25–60 |
| 6 | 60–100 |

Figure 1: Site map of Graz with the city limits, the river Mur crossing the city from north to south, the motorways and the 11 exposition sites




## Results and Discussion

The leaves of tobacco, *Nicotiana tabacum* 'BelW3' showed the typical leaf necroses due to ozone (HEGGESTAD 1991, VDI 2000). The characteristic bifacial lesions were clearly defined areas on the leaves and were identical on the upper and the lower leaf surface. The lesions were characterized by a clear border between turgescent epidermal cells of the healthy parts of the leave and collapsed cells of the lesions (Fig. 2). They are due to lyses of parenchymatic cells and as a consequence both epidermal cell layers were close together (GÜNTHARDT-GOERG 1996).

Distinct age dependence in the development of the lesions could be observed with only slight damage of the young leaves and more severe damage of the older leaves (Tab. 2).

The reliability of the evaluation of visible symptoms on tobacco leaves was confirmed by computer-aided image analysis. Percentage of injury was visually estimated and classified. The leaves were than scanned and analysed using image analysis software, the results were also classified into damage classes. Visual estimation of leaf damage was sufficiently accurate ($n = 73$, Spearman $R = 0.76$, $p < 0.001$). Colour information was absolutely essential for correct threshold settings using the image analysis software. Using black and white images only visual estimation was not in good agreement with computer analysis (DELLA MEA & al. 1997). Visual estimation is much less time consuming and it will not be replaced by image analysis methods in the near future. However, for the purpose of standardization a uniform, simple and reliable method based on image analysis would be very welcome.

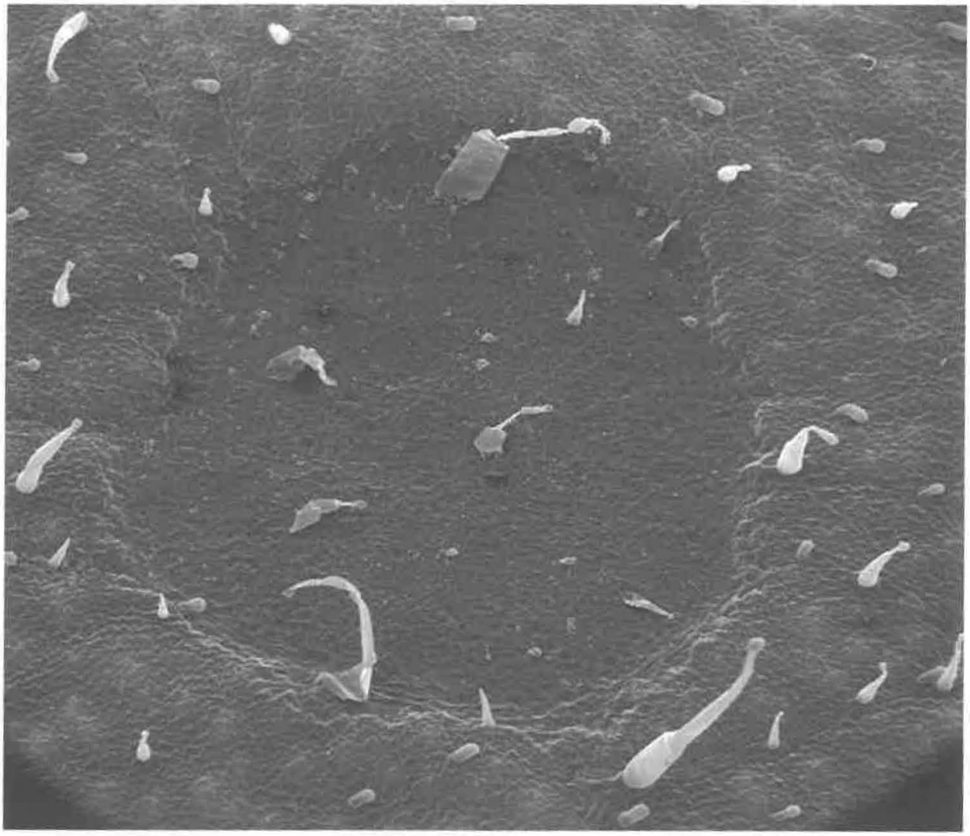


Figure 2: SEM micrograph of a leaf of *Nicotiana tabacum* 'BelW3'; characteristic lesion due to ozone; horizontal diameter of the lesion = 1.85 mm

Table 2: Dependence of damage class (*Nicotiana tabacum* 'BelW3') on the leaf age; mean ± standard deviation; 1 = oldest leaf, 9 = youngest leaf.

| Age class | n | Damage class |
|---|---|---|
| 1 | 110 | 2.29 ± 1.32 |
| 2 | 323 | 2.60 ± 1.26 |
| 3 | 455 | 2.50 ± 1.40 |
| 4 | 489 | 2.00 ± 1.43 |
| 5 | 489 | 1.35 ± 1.43 |
| 6 | 462 | 0.82 ± 1.15 |
| 7 | 392 | 0.44 ± 0.83 |
| 8 | 309 | 0.18 ± 0.50 |
| 9 | 201 | 0.11 ± 0.39 |

At sites 1, 3, 5 and 6 (Fig. 1) tobacco plants were exposed at sites where also continuous ozone measurements were performed. The mean damage class per site was correlated with the AOT 40 value (ppb.h) of the exposition period (14 days) for all evaluation periods (Fig. 3). A high ozone dose corresponded with high leaf damage as was also reported from some other studies (Krupa & al. 1993, Kostka-Rick 2002). However, in spite of average ozone concentrations in beginning of May the leaves only showed below-average damage (box of 4 values in Fig. 3). Obviously, the reaction mechanism in developing leaf necroses also depends on other factors and not only ozone concentrations. Vapour pressure deficit is one important environmental factor in modulating the ozone response (Benton & al. 2000).

During the whole exposition period the typical leaf necroses could be observed depending on the site. Highest damage on single leaves (damage class 6) occurred in August. The overall results for the whole exposition period can be seen in Tab. 3.

At the same time tobacco plants were exposed in a slightly different methodological approach (exposition on the ground, exposure time: 4 weeks) within the scope of an international project with participation of eight European cities (Dugauquier & al. 1997, Stabentheiner & al. 2002). The lowest leaf damage due to ozone could be observed in the industrialized cities Lille (France) and Charleroi (Belgium) and highest leaf damage in the Italian cities Modena, Bologna and Florenz. The results of Graz were comparable to that of Nürnberg (Germany) and Tampere (Finland) and ranged in the centerfield (Dugauquier & al. 1997).

Table 3: Damage class of tobacco varieties BelW3 and BelB of all sites (compare Fig. 1) for the whole exposition period (14-days exposure, beginning of May till end of September); mean ± standard deviation

| Site | BelW3 | BelB |
|---|---|---|
| 1 | 1.44 ± 0.46 | 0.31 ± 0.23 |
| 2 | 1.11 ± 0.57 | 0.22 ± 0.13 |
| 3 | 1.53 ± 0.59 | 0.29 ± 0.20 |
| 4 | 0.90 ± 0.39 | 0.32 ± 0.15 |
| 5 | 1.05 ± 0.44 | 0.24 ± 0.12 |
| 6 | 1.11 ± 0.47 | 0.15 ± 0.07 |
| 7 | 1.20 ± 0.56 | 0.23 ± 0.14 |
| 8 | 1.21 ± 0.36 | 0.27 ± 0.12 |
| 9 | 1.07 ± 0.38 | 0.24 ± 0.11 |
| 10 | 1.44 ± 0.62 | 0.30 ± 0.16 |
| 11 | 1.08 ± 0.50 | 0.21 ± 0.06 |

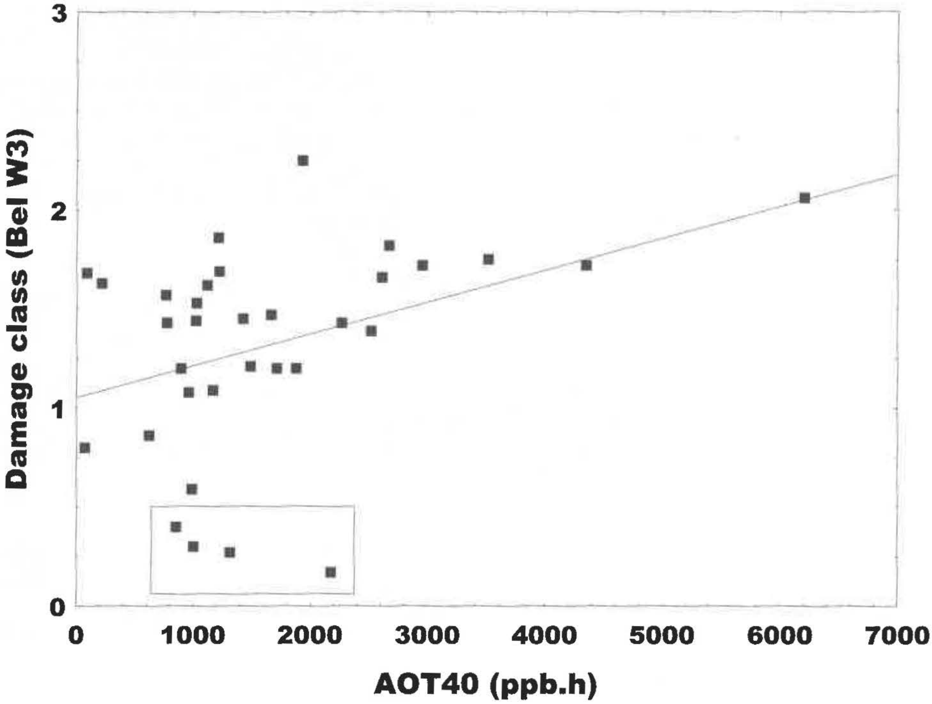



Figure 3: Scatter plot of AOT 40 values of ozone (ppb.h; 14 days) against damage class of *Nicotiana tabacum* 'BelW3'; n = 33, Spearman R = 0.422, p = 0.014; the marked box represents the mean damage class values of the 4 sites for the first exposition period beginning middle of May; correlation without these values: n = 29, Spearman R = 0.51, p = 0.005.

Though the differences between the exposition sites in the city area are not very distinct (Tab. 3) it was possible to identify areas differing in ozone-depending damage. The highest damage class could be observed at sites on the elevated outskirts in the east (10) and northeast (3) of Graz. The plants on the Schloßberg (1), an elevated site near the city centre also showed high leaf damages. The sites with the lowest leaf damage due to ozone (4, 5, and 9) were situated near main traffic routes. The damage on leaves of BelB was always very low and no distinct site differences could be observed.

## Conclusions

The tobacco cultivar BelW3 is a sensitive and reliable bioindicator for ozone. Though the indicator is very sensible its reaction is representative also for the reaction of native plants (BUNGENER & al. 1999). It is possible to identify the spatial and temporal ozone distribution within a project area. A strict compliance with guidelines (e.g., VDI 2000) and the further development of standardized evaluation methods using computer-aided image analysis will increase the acceptance at a national and international level.

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